View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Digital Repository @ Iowa State University

IOWA STATE UNIVERSITY
Digital Repository

Animal Science Publications

Animal Science

11-1-1997

Major histocompatibility complex class II DNA polymorphisms in chicken strains selected for Marek's disease resistance and egg production or for egg production alone

N. Lakshmanan
Iowa State University

J. S. Gavora
Iowa State University

Susan J. Lamont
Iowa State University, sjlamont@iastate.edu

Follow this and additional works at: https://lib.dr.iastate.edu/ans_pubs

 Part of the Agriculture Commons, Genetics Commons, and the Poultry or Avian Science Commons

The complete bibliographic information for this item can be found at https://lib.dr.iastate.edu/ans_pubs/821. For information on how to cite this item, please visit http://lib.dr.iastate.edu/howtocite.html.

This Article is brought to you for free and open access by the Animal Science at Iowa State University Digital Repository. It has been accepted for inclusion in Animal Science Publications by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.

Major histocompatibility complex class II DNA polymorphisms in chicken strains selected for Marek's disease resistance and egg production or for egg production alone

Abstract

The objective of this study was to investigate frequencies of major histocompatibility complex (MHC) class II restriction fragments in two groups of White Leghorn strains. Each group consisted of an unselected control, a strain selected for egg production traits, and a strain selected for egg production traits and Marek's disease (MD) resistance. PvuII-digested genomic DNA was hybridized with a chicken genomic MHC class II probe. The MHC class II DNA fragment frequencies in the selected strains differed from those in the related unselected control and in the strain selected using the same criteria from a different base population. Based on the sizes of the breeding populations, particularly those in the control strain and in the strain selected for egg production, it was considered unlikely that the observed changes of the MHC class II fragment frequencies were due to random genetic drift. The data suggested that some MHC class II bands are associated with production traits or with MD resistance, and that these associations tend to be unique to each genetic background. Hence, MHC class II genes are likely candidates for the investigation of quantitative trait loci in egg production and disease resistance traits such as those for which the studied strains were selected.

Keywords

Major Histocompatibility Complex class II, selection, strain, egg production, Marek’s disease resistance

Disciplines

Agriculture | Animal Sciences | Genetics | Poultry or Avian Science

Comments

This article is published as Lakshmanan, N., J. S. Gavora, and S. J. Lamont. "Major histocompatibility complex class II DNA polymorphisms in chicken strains selected for Marek's disease resistance and egg production or for egg production alone." *Poultry Science* 76, no. 11 (1997): 1517-1523. DOI: 10.1093/ps/76.11.1517. Posted with permission.

Creative Commons License

This work is licensed under a Creative Commons Attribution-Noncommercial-No Derivative Works 4.0 License.

This article is available at Iowa State University Digital Repository: https://lib.dr.iastate.edu/ans_pubs/821

IMMUNOLOGY

Major Histocompatibility Complex Class II DNA Polymorphisms in Chicken Strains Selected for Marek's Disease Resistance and Egg Production or for Egg Production Alone[1]

N. LAKSHMANAN,* J. S. GAVORA,† and S. J. LAMONT*,[2]

*Department of Animal Science, Iowa State University, Ames, Iowa 50011-3150 and †Centre for Food and Animal Research, Agriculture and Agri-Food Canada, Ottawa, Ontario, K1A 0C6, Canada

ABSTRACT The objective of this study was to investigate frequencies of major histocompatibility complex (MHC) class II restriction fragments in two groups of White Leghorn strains. Each group consisted of an unselected control, a strain selected for egg production traits, and a strain selected for egg production traits and Marek's disease (MD) resistance. *Pvu*II-digested genomic DNA was hybridized with a chicken genomic MHC class II probe. The MHC class II DNA fragment frequencies in the selected strains differed from those in the related unselected control and in the strain selected using the same criteria from a different base population. Based on the sizes of the breeding populations, particularly those in the control strain and in the strain selected for egg production, it was considered unlikely that the observed changes of the MHC class II fragment frequencies were due to random genetic drift. The data suggested that some MHC class II bands are associated with production traits or with MD resistance, and that these associations tend to be unique to each genetic background. Hence, MHC class II genes are likely candidates for the investigation of quantitative trait loci in egg production and disease resistance traits such as those for which the studied strains were selected.

(*Key words*: Major Histocompatibility Complex class II, selection, strain, egg production, Marek's disease resistance)

1997 Poultry Science 76:1517–1523

INTRODUCTION

Marek's disease (MD) is a lymphoproliferative disease caused by a DNA virus of the herpes virus group. All chickens appear to be susceptible to infection with MD virus. Although several factors play a role in the outcome of MD virus infections (Calnek, 1985), the genetic makeup of the chicken greatly influences the incidence and pathogenesis of the disease. Three principal mechanisms of genetic resistance are recognized: resistance associated with the MHC, resistance associated with MHC-like genes (*Rfp*-Y), and resistance due to variation in susceptibility of T cells to transformation (Bacon, 1987; Payne, 1990; Wakenell *et al.*, 1996).

The chicken MHC, also known as the *B*-complex (*Ea-B*), was originally described as a blood group system (Briles *et al.*, 1950) and later identified as the MHC (Schierman and Nordskog, 1961). The chicken MHC is associated with resistance to bacterial, parasitic, and viral diseases and other important economic traits in chickens (Bacon, 1987; Lamont, 1989; Gavora, 1990). Differences in MHC allelic frequencies between strains selected for egg production traits and their control populations have been reported in independent selection programs (Simonsen *et al.*, 1982; Gavora *et al.*, 1986; Lamont *et al.*, 1987). Numerous studies have shown an association of the MHC with Marek's disease resistance (Bacon, 1987).

The MHC of the chicken is composed of three classes of genes, *B-F* (class I), *B-L* (class II), and *B-G* (class IV). Briles *et al.* (1983) reported that MHC-associated resistance to MD is mapped to the *B-F* region rather than to *B-G* region. With the present level of understanding of chicken MHC genomic organization (Guillemot *et al.*, 1989b), it is probable that the serologically identified *B-F* region in the Briles *et al.* (1983) study contained class I and class II genes. Genes associated with Marek's disease resistance were also localized in *B-F*/*B-L* by Hepkema *et al.* (1993). Because of the lack of generally available *B-F*/*B-L* recombinant lines, the specific association of *B-L* region genes with resistance to Marek's disease is unknown.

Recently, Briles *et al.* (1993) and Miller *et al.* (1994) described a set of MHC-like class I and class II genes known as "restriction fragment polymorphism pattern-Y" (*Rfp*-Y) among pedigreed families. Although both the *B*-complex and *Rfp*-Y are physically assigned to a

Received for publication February 10, 1997.
Accepted for publication June 22, 1997.
[1]This is Journal Paper Number J-16069 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA; Project Numbers 2237 and 2351; and CFAR contribution Number 2284.
[2]To whom correspondence should be addressed.

microchromosome (chromosome 16), no genetic linkage between the *B*-complex and *Rfp*-Y was observed (Briles *et al.*, 1993). This genetically independent, polymorphic MHC-like system contains at least two MHC class I genes and three MHC class II genes (Miller *et al.*, 1994, 1996).

The MHC class I and class II antigens are involved in the presentation of antigenic peptides to the immune system and are also involved in T cell repertoire selection (Klein, 1986). Therefore, class II genes, both as candidate genes and as loci closely linked to other immune-function genes in the MHC, are potential markers in selection for disease resistance. In this study, we determined the frequency distribution of MHC class II restriction fragments in chicken strains selected for egg production traits or for production traits and Marek's disease resistance in two different genetic backgrounds. Our hypothesis was that if MHC class II antigens influenced the traits under selection, the frequencies of restriction fragments would differ among the control, production-selected, and resistance-selected strains. Even though the differences found among the strains were not always consistent between the two sets of strains examined, the results confirmed the hypothesis and provide a justification for the inclusion of MHC class II genes as candidate genes in the search for the quantitative trait loci underlying egg production and disease resistance characters.

MATERIALS AND METHODS

Experimental Birds

Birds from six White Leghorn strains were used. The strains were classified, based on their origin, into two groups. Each group consisted of an unselected randombred control strain, a strain selected for high egg production, fertility, hatchability, egg size, and egg quality traits (the "production-selected strain"), and a strain selected for resistance to Marek's disease (MD) plus the aforementioned traits (the "resistance-selected strain") (Gavora, 1979; Gavora *et al.*, 1986; Gowe and Fairfull, 1990). In Group A, the unselected control Strain 5 originated from a narrow genetic base in Ottawa in 1950, Strain 1 was a production-selected strain, and Strain 3R was a resistance-selected strain. In Group B, the randombred control Strain 7 was synthesized from four North American commercial stocks in 1960, Strain 8 was a production-selected strain, and Strain 8R was a resistance selected strain from this population base. The breeding populations of the control and production-selected strains were sizeable (Table 1), and within these strains, designated breeders were mated at random, avoiding matings of full- and half-sibs, and thus reducing inbreeding. To produce the resistance-selected strains, a series of about 40 inbred lines was derived by son-mother mating in each of six generations from each of the production-selected strains, Strains 1 and 3 in Group A and Strains 8 and 9 in Group B. Strains 1 and 9 were similar to Strains 3 and 8, respectively, in their origin and selection but were not used in this study. After three generations of full- and half-sib matings with between- and within-line selection for the egg production traits and MD resistance, the selected inbred lines were pooled to form the resistance-selected strains. The formation of the resistance-selected strains was completed in 1979 and 14 such inbred lines were incorporated into Strain 3R and 19 into Strain 8R. The response to MD virus challenge varied greatly among the selected and unselected control strains. Population means for production traits, MD incidence, and mortality for the control and selected strains are presented in Table 2 (modified from Ameli *et al.*, 1992).

DNA Isolation, Restriction Enzyme Digestion, Electrophoresis, and Transfer of DNA

Genomic DNA from whole blood samples was isolated using a standard protocol (Dunnington *et al.*, 1990). An initial experiment was conducted to determine the most informative (polymorphic and unambiguously scorable autoradiographic bands) single- or double-restriction enzyme digest for use in the present study. Genomic DNA isolated from whole blood of birds from six different serologically defined MHC haplotypes (*Ea-B* 2/2, 12/12, 13/13, 15/15, 19/19, 21/21) and restriction enzymes[3] *Bam*HI, *Hin*dIII, *Pvu*II, and *Taq*I were used. On the basis of the results obtained (Table 3) and previous reports (Warner *et al.*, 1989; Emara *et al.*, 1992), *Pvu*II was chosen for subsequent use in this study. Ten to 2 μg of genomic DNA were digested with *Pvu*II restriction endonuclease (4 U/μg of DNA) overnight at 37 C. Eight micrograms of restriction enzyme-digested DNA per sample were size-separated on 0.8% agarose gels with 1 TBE (0.09 *M* Tris-borate, 0.002 *M* EDTA) buffer for 48 h at 36 V (1.3 V/cM). The DNA was then transferred (Southern, 1975) to nylon membranes (Hybond-N^+)[4] with 20 SSC for 16 to 20 h. The DNA fragments were fixed on the nylon membrane by placing the membrane on a 0.4 *N* NaOH saturated Whatman paper[5] for 10 to 15 min followed by a quick rinse with 5 SSC. As a reference standard, a *Pvu*II-digested DNA pool, representing the six different MHC haplotypes of the experimental population, was included on each gel. Samples were loaded such that none was more than three lanes away from a *Hin*dIII and *BSTE*II digested nonradiolabeled lambda DNA molecular weight marker lane.

Hybridization and Autoradiography

Membranes were prehybridized with a buffer that contained a 0.263 *M* sodium phosphate, 7% SDS, 1 m*M* EDTA, 1% BSA, and salmon sperm DNA (100 μg/mL) for

[3]Promega, Madison, WI 53711.
[4]Amersham Corp., Arlington Heights, IL 60005.
[5]Fisher Scientific, Itasca, IL 60143.

TABLE 1. Description of the White Leghorn strains used in this study

Group	Strain	Origin	Year		Selection criteria	Number of breeders per generation during				Inbreeding coefficient in 1980
						Selection		Maintenance		
			Strain derived	Selection terminated		Sires	Dams	Sires	Dams	
A	5	Narrow genetic base population in Ottawa	1950		None			80	240	6.3[1]
	1	Strain 5	1950	1980	Egg production traits	28	224	40 to 50	100 to 160	22.8[1]
	3R	Strains 1 and 3	1970	1979	Egg production traits and resistance to Marek's disease	Inbred lines (see text for details)		50	100	18.3[2]
B	7	Four North American commercial stocks	1960		None			80	240	2.4[1]
	8	Strain 7	1969	1980	Egg production traits	28	224	40 to 50	100 to 160	9.9[1]
	8R	Strain 8 and 9	1970	1979	Egg production traits and resistance to Marek's disease	Inbred lines (see text for details)		50	100	6.3[2]

[1]Gowe and Fairfull, 1980; Gowe *et al.*, 1993.
[2]J. S. Gavora, unpublished results.

TABLE 2. Population[1] means for production traits and for Marek's disease (MD) incidence and mortality in MD challenge tests with two very virulent strains of MD virus (BC1 and RB-1B)[2]

Strain	Age at first egg	Egg production to 497 d of age	Egg weight at 2,480 d	MD incidence				MD mortality			
				BC1		RB-1B		BC1		RB-1B	
				Male	Female	Male	Female	Male	Female	Male	Female
	(d)	(no.)	(g)	(%)							
Group A											
5[3]	169.3	244.3	50.2	6.8	31.2	79.3	79.6	3.4	10.9	63.5	70.4
1[4]	152.1	287.2	55.0	0.0	21.6	60.0	83.3	0.0	10.8	27.5	40.0
3R[5]	155.7	271.9	56.6	4.7	0.0	34.2	34.2	2.4	0.0	5.7	11.4
Group B											
7[3]	165.1	250.8	51.7	12.9	18.3	50.9	68.4	7.4	5.6	33.0	48.0
8[4]	151.8	272.0	56.7	0.0	3.0	20.0	29.0	0.0	0.0	11.4	19.4
8R[5]	154.4	275.5	56.6	0.0	0.0	8.3	12.1	0.0	0.0	2.8	3.0

[1]The population size for egg production tests was 120 birds per strain and for challenge tests was between 67 to 75 for selected strains and between 115 and 125 for each control strain.
[2]Modified from Ameli *et al.* (1992).
[3]Unselected control.
[4]Production-selected strain.
[5]Resistance-selected strain.

TABLE 3. Restriction enzyme efficacy of detecting chicken MHC class II polymorphism in restriction fragment length polymorphism (RFLP) analysis of six different MHC haplotypes (*Ea-B* 2/2, 12/12, 13/13, 15/15, 19/19, and 21/21)

	Restriction fragments (bands)				
Enzyme	Number per individual	Number monomorphic bands	Number polymorphic bands	Band resolution	Number of patterns
*Pvu*II	4 to 6	3	5	Good	4
*Pvu*II + *Taq*I	3 to 6	4	4	Fair	4
*Pvu*II + *Hin*dIII	3 to 4	2	4	Fair	4
*Pvu*II + *Bam*HI	3 to 4	3	3	Fair	4
*Taq*I	4 to 5	4	1	Good	3
*Taq*I + *Bam*HI	3 to 5	3	3	Good	3
*Taq*I + *Hin*dIII	4 to 5	2	5	Good	3
*Hin*dIII	4	4	0	Good	1
*Hin*dIII + *Bam*HI	4	4	0	Good	1
*Bam*HI	4 to 5	2	5	Good	3

60 to 90 min at 65 C. This procedure was followed by an addition of 50 ng of ^{32}P-deoxycytidine triphosphate (dCTP) labeled (random priming method as described by Feinberg and Vogelstein, 1983) chicken MHC class II probe (CCII-7-1; Xu *et al.*, 1989), which contains genomic sequences of the β-1, β-2, transmembrane and cytoplasmic portion of the MHC class II β chain. The membranes were allowed to hybridize overnight at 65 C and were washed twice with 0.263 *M* sodium phosphate, 1% SDS (20 min) at 65 C, followed by two washes (20 min each) with 2 SSC and 0.1% SDS, two washes (20 min each) with 1 SSC and 0.1% SDS, and two washes (20 min each) with 0.5 SSC and 0.1% SDS. Membranes were autoradiographed for 2 to 3 d with Fuji-RX x-ray[6] film at –70 C with intensifying screens.[7]

Estimation of DNA Fragment Molecular Weight

The molecular weights of sample restriction fragment length polymorphism (RFLP) fragments were estimated from their migratory distance on the basis of the migratory distance and molecular weight of the molecular weight marker bands (Weir, 1990). Nine bands of different molecular weights were identified among experimental birds and these were assigned numbers based on molecular weight. The same band numbers were used across strains to identify DNA fragments that appeared to have similar molecular weights among all strains studied.

Statistical Analysis

Band frequency data were analyzed within groups (A and B) by chi-square tests with 2 3 contingency tables to test the independence of strain and each band presence. Data from Groups A and B were also analyzed for band frequency differences between unselected control strains (5 vs 7), between production-selected strains (1 vs 8), and between resistance-selected strains (3R vs 8R).

RESULTS

The MHC class II band frequencies of all strains are listed (Table 4) and representative patterns are illustrated (Figure 1). The two unselected control Strains 5 and 7 significantly differed ($P < 0.05$) for several band frequencies. The frequencies of MHC class II bands 2 and 6 were significantly lower, and bands 7 and 9 were significantly higher, in Strain 5 than in Strain 7. Thus, the original MHC haplotype frequencies in the control

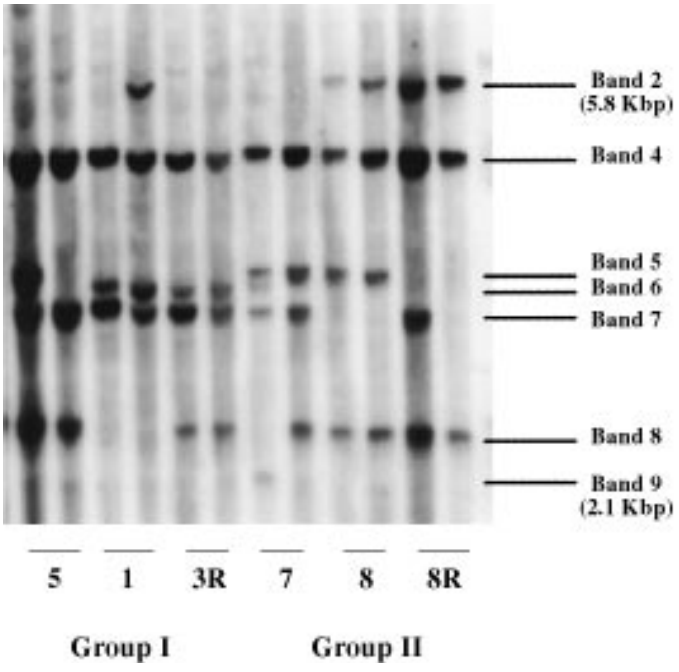

FIGURE 1. Autoradiograph representative of hybridization patterns produced by chicken MHC class II genomic probe and DNA from strains (numbered on lanes) used in this study.

[6]Fuji Photo Film Co., Ltd., Japan.
[7]Dupont, Wilmington, DE 19898.

TABLE 4. Frequency distribution of MHC class II related bands among control strains (5 and 7), production-selected strains (1 and 8), and resistance-selected strains (3R and 8R) from two different genetic backgrounds (Group A and B)

Band number	Estimated molecular weight	Group A			Group B		
		5 (n = 36)	1 (n = 34)	3R (n = 33)	7 (n = 57)	8 (n = 23)	8R (n = 35)
	(kbp)						
1	6.5	0.00	0.00	0.00	0.04	0.00	0.00
2	5.8	0.19[a]	0.62[b]	0.22[a]	0.55	0.78	0.69
3	5.5	0.00	0.00	0.00	0.09	0.00	0.00
4	4.4	0.97	1.00	1.00	0.96	1.00	0.97
5	3.1	0.42[a]	0.00[b]	0.00[b]	0.46[a]	0.61[a]	0.03[b]
6	2.8	0.03[a]	0.56[b]	0.55[b]	0.33[a]	0.04[b]	0.00[b]
7	2.5	0.89	0.88	1.00	0.58[a]	0.17[b]	0.63[a]
8	2.4	0.94	0.85	0.97	0.93	1.00	1.00
9	2.1	0.33[a]	0.00[b]	0.00[b]	0.00	0.00	0.00

[a,b]Values in a row within each group with no common superscript differ significantly (P 0.05).

strains representing the original genetic base populations were quite different.

In comparing the production-selected and control strains of Group A, bands 2 and 6 were present in higher frequencies ($P < 0.05$), and band 5 in lower frequency ($P < 0.05$), in the production-selected Strain 1 than in the unselected control Strain 5. In Group B, two MHC class II bands (bands 6 and 7) were present in lower frequencies ($P < 0.05$) in the production-selected Strain 8 than in the control Strain 7.

In comparing the MD-selected strain with the control strain of Group A, MHC class II band 6 was more frequent, and band 5 was less frequent, in resistance-selected Strain 3R than in control Strain 5. Within Group B, MHC class II bands 5 and 6 were at significantly lower ($P < 0.05$) frequencies in resistance-selected Strain 8R than in control Strain 7. The MHC class II band 5 frequency was lower, and band 7 frequency was higher, in Strain 8R than in production-selected Strain 8.

DISCUSSION

In analyzing the frequencies of MHC class II DNA restriction fragments among two sets of control, production-selected, and production plus resistance-selected strains of White Leghorn chickens, significant differences in several MHC class II band frequencies were detected among the strains within each group. This is in agreement with previous studies on the same strains (Gavora *et al.*, 1986), in which the frequency of birds with serologically defined MHC alleles, B^{21} and B^{2}, was significantly higher in production-selected Strain 8 and resistance-selected Strain 8R than in unselected control Strain 7.

The difference in the MHC class II DNA fragment frequencies in this study between the unselected control and production-selected strains can be viewed as being primarily associated with the selection for the production traits. Because the resistance-selected lines had resistance selection superimposed on production selection (Table 1), the differences in the frequencies between the resistance-selected and production-selected strains can similarly be considered mainly associated with the selection for Marek's disease resistance. In this context, it may be useful to note that unlike control Strain 5, which was kept unselected since 1950, Strain 7, which formed the genetic base for Group B, was created from commercial stocks (Table 1). Hence, not only was the Strain 5 genetic base substantially different from that of Strain 7, but Strain 7 frequencies of the MHC class II DNA fragments should be viewed as having been influenced by the selection for production traits and viability practiced by the breeding companies from which the commercial stocks originated.

The data obtained in this study reveal the following possible indications of associations between the frequencies of the MHC class II DNA fragments and the traits under selection (Table 4). Band 2 frequency was reduced during the resistance selection in Group A relative to its production-selected counterpart, Strain 1. Hence, it may be associated with MD susceptibility. Band 5 frequency was significantly reduced during both production selection and resistance selection ($P < 0.05$) in Group A. In Group B, band 5 frequency was not significantly altered during the production selection, however, band 5 frequency was reduced during resistance selection ($P < 0.05$). This pattern may also be indicative of a positive relationship with MD resistance. The cause of band 5 maintenance at control-level frequencies in the production-selected Strain 8 is unclear, especially given its dramatic reduction in all other selected lines of both groups. Band 7 frequency was kept at high frequency in all strains of Group A and was increased, relative to its production-only selected counterpart line, during the resistance selection in Group B ($P < 0.05$). Band 8 remained at high frequencies in all six strains. Thus it seems likely that these latter two bands do not have a negative relationship with MD resistance. Finally, because band 9 frequency was reduced under production selection, kept low by MD selection, and was absent in

Group B, the band may not have a favorable association with resistance to MD. The above indications of possible associations are rather weak. Therefore, the results should be considered preliminary and used with the usual caution.

There was inconsistency in the pattern of changes observed in band 6, the frequency of which was significantly ($P < 0.05$) increased with production selection in Group A and significantly ($P < 0.05$) decreased with similar selection in Group B. Bands 1 and 3 were not informative, as they were found at low frequencies or were absent in the strains studied.

Given the observed inconsistency in the frequency changes of some MHC class II DNA restriction fragments between lines selected for similar phenotypic traits from two different genetic backgrounds, the question arises as to whether the observed changes in this study could be a result of genetic drift. The possibility that some of the changes are indeed due to drift can not be dismissed outright. However, examination of the sizes of the breeding populations (Table 1), particularly those in the control and production-selected strains, indicates that it is not likely that genetic drift played a significant role in the observed changes. Furthermore, the techniques for the maintenance of the unselected control strains were designed to minimize the genetic drift (Gowe *et al.*, 1959) so that the fragment frequencies observed in the control strains are expected to closely represent the situation at the time the production-selected strains were derived. The possibility that genetic drift influenced the MHC class II DNA fragment frequencies in the resistance-selected strains is somewhat greater because the technique for their formation included derivation and subsequent recombination of multiple inbred lines as previously described.

The choice of the restriction enzyme used to analyze MHC class II polymorphism was based on the initial study in which *Pvu*II enzyme digestion of DNA from six different MHC haplotypes of Strain 7 gave more polymorphic and unambiguously scorable bands than *Bam*HI, *Hin*dIII, and *Taq*I single or double restriction enzyme digests (Table 3) and previous studies (Warner *et al.*, 1989; Emara *et al.*, 1992). Perhaps other enzyme-MHC class II probe combinations would be better for comparisons of MHC class II bands in strains from different genetic origins. The chicken MHC class II probe used to analyze the genomic DNA was a genomic probe (2.3 kbp) coding for the MHC class II β chain. Several MHC class II genes have been cloned (Zoorob *et al.*, 1993), and at least two of them are expressed (Pharr *et al.*, 1993). Because of the complex nature of the chicken MHC gene organization and size, analysis of genomic DNA with other MHC probes might disclose additional associations between MD resistance and MHC genes. Also, the probe used in this study may have revealed polymorphisms attributable to adjacent MHC genes, rather than class II alone. The MHC class I genes and other nonclassical MHC genes, such as C12.3 (which encodes for a G-protein-like molecule), are potential candidate genes for future molecular studies. Differences in C12.3 structure or expression have been proposed to be associated with genetic variability in lymphocyte activation and resistance to Marek's disease (Guillemot *et al.*, 1989a).

Recent identification of the MHC-like genes, the *Rfp*-Y genes, by Briles *et al.* (1993) and Miller *et al.* (1994) and studies of their role in MD resistance (Bacon *et al.*, 1996; Wakenell *et al.*, 1996) have evoked an interest among investigators. Because there is high sequence similarity between genes of the *B*-complex and *Rfp*-Y, the genomic MHC class II probe used in this study might detect class II genes from both the classical MHC as well as from *Rfp*-Y. A study with an *Rfp*-Y linked or specific probe, therefore, would aid in discrimination of the bands from these two different chromosomal regions.

The selection for the egg production traits and MD resistance practiced in the strains examined both involved multiple polygenic traits, so that the selection likely influenced a significant proportion of the genome. The finding of this study that the frequencies of MHC class II DNA fragments in several instances differed among the strains, strongly suggest that MHC class II genes are involved in the polygenes, even though some of the observed frequency differences may be because of genetic drift. The degree of influence of these genes on the polygenes is, at the moment, impossible to assess. Nevertheless, the results provide a basis for the inclusion of MHC class II genes among candidate genes for the investigation of the genetic architecture of egg production and disease resistance polygenes of the domestic chicken.

ACKNOWLEDGMENT

The excellent technical assistance of S. Hoffman is gratefully acknowledged.

REFERENCES

Ameli, H., J. S. Gavora, J. L. Spencer, and R. W. Fairfull, 1992. Genetic resistance to Marek's disease viruses and its relationship to production traits in chickens. Can. J. Anim. Sci. 72:213–225.

Bacon, L. D., 1987. Influence of the major histocompatibility complex on disease resistance and productivity. Poultry Sci. 66:802–811.

Bacon, L., R. Vallejo, H. Cheng, and R. Witter, 1996. Failure of Rfp-Y genes to influence resistance to Marek's disease. Pages 69–74 *in*: Current Research on Marek's Disease. R. F. Silva, H. H. Cheng, P. M. Coussens, L. F. Lee, and L. F. Velicer, ed. America Association of Avian Pathologists, Kennett Square, PA.

Briles, W. E., W. H. McGibbon, and M. R. Irwin, 1950. On multiple alleles affecting cellular antigens in the chicken. Genetics 35:633–652.

Briles, W. E., R. W. Briles, R. E. Taffs, and H. A. Stone, 1983. Resistance to malignant lymphoma in chickens is mapped to subregion of Major Histocompatibility (B) complex. Science 219:977–979.

Briles, W. E., R. M. Goto, C. Auffray, and M. M. Miller, 1993. A polymorphic system related to but genetically independent of the chicken major histocompatibility complex. Immunogenetics 37:408–414.

Calnek, B. W., 1985. Genetic resistance. Pages 293–328 *in*: Marek's Disease, Scientific Basis and Methods of Control. L. N. Payne, ed. Martinus Nijhoff Publishing, Boston, MA.

Dunnington, E. A., O. Gal, Y. Plotsky, A. Haberfeld, T. Kirk, A. Goldberg, U. Lavi, A. Cahaner, P. B. Siegel, and J. Hillel, 1990. DNA fingerprints of chickens selected for high and low body weight for 31 generations. Anim. Genet. 21: 247–257.

Emara, M. G., K. E. Nestor, D. N. Foster, and S. J. Lamont, 1992. The turkey major histocompatibility complex: identification of class II genotypes by restriction fragment length polymorphism analysis of deoxyribonucleic acid. Poultry Sci. 71:2083–2089.

Feinberg, A. P., and B. Vogelstein, 1983. A technique of radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132:6–13.

Gavora, J. S., 1979. Genetic techniques for controlling Marek's disease and for improvement of multiple economic traits in high egg production chickens. Pages 32–57 *in*: Proceedings of the 18th National Breeders' Round Table, Memphis, TN. Poultry Breeders of America, Tucker, GA.

Gavora, J. S., 1990. Disease genetics. Pages 805–846 *in*: Poultry Breeding and Genetics. R. D. Crawford, ed. Elsevier, New York, NY.

Gavora, J. S., M. Simonsen, J. L. Spencer, R. W. Fairfull, and R. S. Gowe, 1986. Changes in the frequency of major histocompatibility haplotypes in chickens under selection for both high egg production and resistance to Marek's disease. J. Anim. Breed. Genet. 103:218–226.

Gowe, R. S., and R. W. Fairfull, 1980. Performance of six long-term multi-trait selected Leghorn strains and three control strains and a strain-cross evaluation of the selected strains. Pages 141–162 *in*: Proceedings of the South Pacific Poultry Science Convention. Auckland, NZ.

Gowe, R. S., and R. W. Fairfull, 1990. Genetic controls in selection. Pages 935–954 *in*: Poultry Breeding and Genetics. R. D. Crawford, ed. Elsevier, New York, NY.

Gowe, R. S., R. W. Fairfull, I. McMillan, and G. S. Schmidt, 1993. A strategy for maintaining high fertility and hatchability in a multiple-trait egg stock selection program. Poultry Sci. 72:1433–1448.

Gowe, R. S., A. Robertson, and B.D.H. Later, 1959. Environment and poultry breeding problems. 4. The value of a random bred control strain in a selection study. Poultry Sci. 38:443–462.

Guillemot, F., A. Billault, and C. Auffray, 1989a. Physical linkage of a guanine nucleotide-binding protein-related gene to the chicken major histocompatibility complex. Proc. Natl. Acad. Sci. USA 86:4594–4598.

Guillemot, F., J. F. Kaufman, K. Skjodt, and C. Auffray, 1989b. The major histocompatibility complex in the chicken. Trends Genet. 5:300–304.

Hepkema, B. G., J. J. Blanker, G.A.A. Albert, M.G.J. Tilanus, E. Egberts, A. J. van der Zijpp, and E. J. Hensen, 1993. Mapping of susceptibility of Marek's disease within the major histocompatibility (B) complex by refined typing of White Leghorn chickens. Anim. Genet. 24:283–287.

Klein, J., 1986. Function. Pages 424–607 *in*: Natural History of the Major Histocompatibility Complex. John Wiley and Sons, New York, NY.

Lamont, S. J., 1989. The chicken major histocompatibility complex in disease resistance and poultry breeding. J. Dairy Sci. 72:1328–1333.

Lamont, S. J., Y.-H. Hou, B. M. Young, and A. W. Nordskog, 1987. Differences in major histocompatibility complex gene frequencies associated with feed efficiency and laying performance. Poultry Sci. 66:1064–1066.

Miller, M. M., R. Goto, A. Bernot, R. Zoorob, C. Auffray, N. Bumstead, and W. E. Briles, 1994. Two Mhc class I and Mhc class II genes map to the chicken Rfp-Y system outside the B-complex. Proc. Natl. Acad. Sci. USA 91: 4397–4401.

Miller, M. M., R. Goto, R. L. Taylor, Jr., R. Zoorob, C. Auffray, R. W. Briles, W. E. Briles, and S. E. Bloom, 1996. Assignment of *Rfp*-Y to the chicken microchromosome and evidence for high frequency recombination associated with the nucleolar organizer region. Proc. Natl. Acad. Sci. USA 93:3958–3962.

Payne, L. N., 1990. Marek's disease. Pages 96–105 *in*: Poultry Diseases. 3rd ed. F.T.W. Jordon, ed. Baillieve Tindall, Tokyo, Japan.

Pharr, G. T., H. D. Hunt, L. D. Bacon, and J. B. Dodgson, 1993. Identification of class II major histocompatibility complex polymorphisms predicted to be important in peptide antigen presentation. Poultry Sci. 72:1312–1317.

Schierman, L. W., and A. W. Nordskog, 1961. Relationship of blood type to histocompatibility in chickens. Science 134: 1008–1009.

Simonsen, M., N. Kolstad, L. E. Edfors-Lilja, and P. Sorensen, 1982. Major histocompatibility genes in egg-laying hens. Am. J. Reprod. Immunol. 2:148–152.

Southern, E. M., 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503–517.

Wakenell, P. S., M. M. Miller, R. M. Goto, W. J. Gauderman, and W. E. Briles, 1996. Association between the Rfp-Y haplotype and the incidence of Marek's disease in chickens. Immunogenetics 44:242–245.

Warner, C., B. Gerndt, Y. Xu, Y. Bourlet, C. Auffray, S. Lamont, and A. Nordskog, 1989. Restriction fragment length polymorphism analysis of major histocompatibility complex class II genes from inbred chicken lines. Anim. Genet. 20:225–231.

Weir, B. S., 1990. Molecular data. Pages 222–260 *in*: Genetic Data Analysis: Methods for Discrete Population Genetic Data. Sinauer Associates, Inc., Sunderland, MA.

Xu, Y., J. Pitcovski, L. Peterson, C. Auffray, Y. Bourlet, B. M. Gerndt, A. W. Nordskog, S. J. Lamont, and C. M. Warner, 1989. Isolation and characterization of three class II major histocompatibility complex genomic clones from the chicken. J. Immunol. 142:2122–2132.

Zoorob, R., A. Bernot, D. M. Renoir, F. Choukri, and C. Auffray, 1993. Chicken major histocompatibility complex class II B genes: analysis of interallelic and interlocus sequence variance. Eur. J. Immunol. 23:1139–1145.